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Study on clinical aspects of SPF chickens infected with Ornithobacterium rhinotracheale followed by H9N2 avian influenza

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ABSTRACT

The aim of this study was to characterize the clinical aspects of ORT followed by H9N2 virus in SPF chickens. Forty two one-day-old SPF chicks were divided randomly into two groups (21 chicks in the experimental and 21 chicks in the control group). At the age of three weeks, the chicks in the experimental group were inoculated intratracheally with 1× 10^{10} LD50 of ORT-R87-7/1387, three days later, 1×10^{6} EID50 of A/chicken/Iran/m.1/2010 (H9N2) was administrated intra-ocularly. Each bird in control group was inoculated with sterile PBS intra-ocularly. Chickens in each groups was evaluated from 2 to 14 days post inoculation. The results of this study indicated that the ORT followed by H9N2 virus cannot cause mortality, and only slight clinical signs and gross lesions such depression, ruffled feathers, tracheal congestion and pneumonia were seen in infected chickens.

Key words: H9N2 influenza virus, Ornithobacterium rhinotracheale, SPF chickens, clinical signs

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INTRODUCTION

Avian influenza (AI) is a viral respiratory disease caused by type A influenza viruses, a member of the family Orthomyxoviridea and at present 16 haemaglutinin and 9 neuraminidase subtype have been recognized [2,16,22]. Avian influenza viruses may cause two different diseases on the basis of the severity of clinical signs in poultry [13]. Although earlier pathogenesis studies indicated that the low pathogenic avian influenza viruses (LPAIV) have only cause respiratory and gastrointestinal dysfunction [21], but there were several reports of systemic infections with the LPAI viruses [4,6,7,14]. Some experimental studies show that the H9N2 LPAI virus in SPF chicks was low pathogenic and mortality was not reported [5, 11], but in Asian and middle east countries high mortality rate reported in recent decades [9, 20, 23].

Co-infections of LPAI viruses with other bacteria or viruses also increase mortality rate and exacerbate clinical signs and gross lesions [1, 4, 15, 17, 19, 20, 25].

One of these bacteria in respiratory complex diseases is Ornithobacterium rhinotracheale that co-infection of AI and ORT in a broiler and laying pullet flock was reported for the first time [4]. ORT can be a primary or secondary etiological agent, depending on the strain virulence, environmental factors, the immune status of the host, and the presence of other infectious agents [26].

The objective of this study was to investigate the clinical aspects of ORT followed by H9N2 in SPF White Leghorn chickens as well as clinical signs and gross lesions. Serological response of chickens after infections were also evaluated challenged chicks.

MATERIALS AND METHODS

Bacteria: The Iranian isolate ORT-R87-7/1387 (JF810491) was used in this study. After growing in medium for 48 h at 37°C, the LD50 of ORT was determined to be 1×10^{10}cfu/0.5 ml in broilers.

Virus: The H9N2 subtype of Avian Influenza virus (AIV) A/chicken/Iran/m.1/2010 (H9N2) was used in this study. The virus was passaged 2 times in 11- day -old embryonated eggs by the chorioallantoic route. The dose of inoculum used in birds was 0.1 mL of 1×10^6 mean 50% egg infective dose/mL per bird [18] .

Chickens: Forty two White Leghorn were obtained from the specific pathogen free (SPF) embryonated chicken eggs from Venky's company (Venky's, India), were divided randomly into two groups (21 chicks per group). They were kept in separate positive pressure isolators at Animal Research Unit of Razi Vaccine and Serum Research Institute and received feed and water *ad libitum* during the experiment.

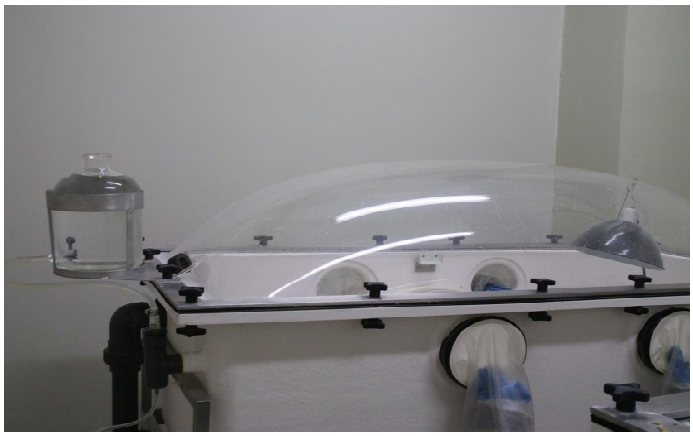

Fig1- positive pressure isolator in this study

Experiments: At the age of 21 days-old, experimental group was inoculated intratracheally with PBS containing 1×10^{10} LD50 of ORT-R87-7/1387 in 0.5 ml, three days later, chorioallantoic fluid containing 1×10^6 EID50 of H9N2 was administrated intra-ocularly in 0.1ml. Each bird in control group was inoculated with 0.1 mL of sterile PBS intra-ocularly. After challenge, all the chickens were monitored daily for clinical signs and mortality. On days 2, 4, 6, 8, 10, 12, 14 and 16 post inoculation (PI), three chickens from each group were randomly selected and were humanely euthanatized and necropsy was performed and gross lesions were recorded.

Samples of different tissues including trachea, thymus, lungs, spleen, liver, kidneys, cecal tonsil, bursa of fabricius and cloaca were aseptically collected for virus detection using RT-PCR technique and ORT was isolated from swab samples [10, 18]. Sera of the birds were collected at the above mentioned days.

Serology

Serum samples were tested for the presence of antibodies to the challenge virus antigen using the HI test .

Statistical analysis

The results obtained from HI test were analyzed by independent t-test. P value less than 0.05 was considered significant.

RESULTS

Infected group Findings

1.Clinical signs:

Some chickens of the infected group showed mild depression at days 3 PI and ruffled feathers and reduced appetite at 4 PI. The clinical signs disappeared at 7 day PI. There was not any mortality in infected group during the experiment.

2.Gross lesions:
The chickens exposed to ORT followed by H9N2 showed the following lesions. Cecal tonsil and kidneys were normal during study period, and there were not any gross pathological changes, but from days 5 PI, in lungs pneumonia and tracheal congestion was seen. Other organs also were normal and there were not any gross pathological changes.

Uninfected control group
There were no clinical signs, gross lesions and mortality in the uninfected control chickens.

Serological findings
HI test was used to measure the antibody titer against H9N2 in the blood samples collected on days 4, 6, 8,10,12,14, and 16 PI. All of serum samples obtained from two groups were negative to AI on day 0, 2, 4 and 6 PI. As shown in Table 1, the mean antibody titer was increased at 8 days PI and reached to 7.0 at 12 days PI in the experimental group. There was no indication of any change in the antibody titer against H9N2 AI virus in the control chickens.

Table1: Antibody titers against avian influenza H9N2 in control and experimental groups (Mean±SE)

	Days PI						
Groups	**4**	**6**	**8**	**10**	**12**	**14**	**16**
Experimental	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}	2.8 ± 0.88^{b}	6.0 ± 0.57^{b}	7.0 ± 0.51^{b}	6.6 ± 0.31^{b}	6.3 ± 0.23^{b}
Control	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}

^{a,b} In each columns, means with different superscripts significantly different ($P<0.05$)

DISCUSSION

The first outbreak of AI in Iranian chicken flocks was reported in 1999 [23].Then widespread outbreaks of virus in commercial broiler chickens was reported by other researcher [14, 15] and since then AI has become an economically important disease in the Iranian poultry industry.

In field and experimental cases of AIV in broiler chickens anorexia, depression, coughing, sneezing, dyspnea and weight losses were reported [3,8,15] and mortality rate were 5% [11] to 20% in experimental studies and 65% in commercial chickens [15], and also in some cases mortality up to 80% was reported in commercial chickens due to concurrent bacterial infections [24]. In some other studies mortality were not reported [1, 7, 9, 22]. Our results indicated that ORT followed by H9N2 could not cause any mortality in SPF chickens and it is different from previous experimental study that report higher mortality in chickens after ORT followed by H9N2 virus inoculation [17], The differences found in this study may be attributed to the type or virulence of the strain of ORT and strain of birds used.

Higher rate of mortality that was reported in some cases possibly because of secondary bacterial or viral infectious as same results was reported by Banani et al (2002), Pan et al (2012) Azizpour et al (2013) which in co-infected broilers mortality rate was increased [1,4, 17].

Previous findings noted that the inoculation of ORT followed by H9N2 virus in the chickens causes ruffled feathers, inactivity and reduced appetite on day 2 PI and respiratory distress, anorexia and emaciation on day 3 PI, they were reported mortality between days 3 and 5 PI. Also in necropsy airsacculitis, pericarditis, peritonitis and scattered areas of haemorrhage in the lungs was reported in broiler chickens infected with ORT followed by H9N2 virus [17]. Our findings indicated that only some depression, ruffled feathers, pneumonia in lungs and tracheal congestion were obvious and this results in agreement with previous reports that ORT followed by H9N2 virus in experimental situation.

The results of serological examination showed antibody increase in H9N2 infected groups and this results was in agreement with previous studies results .[1,3]

CONCLUSION

Our results indicate that ORT followed by H9N2 virus cannot cause mortality, sever clinical signs or gross lesions in SPF chickens.

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